structure between a large and small genome plant is in the size of gene-poor block separating the gene-rich blocks. The 22-fold difference in the size of gene-poor regions compares well with the 35-fold difference in the genome size of wheat and rice. It is, therefore, not surprising that the base pair/centi morgan estimates for the gene cluster regions are nearly comparable in the two species. Thus, it is mainly the presence of substantial amount of nontranscribing repetitive DNA blocks that brings about increase in the genome size of wheat[10]. Further, it has been demonstrated through cytogenetic ladder mapping that the transcribing regions in wheat are present in clusters, and are usually located towards the telomeric ends[11].

Such findings are commensurate with general features of linear differentiation of plant chromosomes that are richly endowed with heterochromatin mainly in telomeric and centromeric regions[12]. Further, the matrix attachment regions (MARs) that serve as link between nuclear membrane attachment sites and expressed sequence tags (ESTs), and facilitate gene expression, remain mainly associated with the subtelomeric regions[13]. Also, it has been shown that even the transformed sequences when targeted to subtelomeric sites show enhanced expression and genetic stability[14,15]. It may, therefore, be considered that the subtelomeric regions are rich in functional genes. Therefore, emphasis may be laid to develop maps for this region as a first step, at least in the selected plants where India has major interest. It is not a very difficult approach, since the techniques for microdissection and microamplification of specific chromosome sequences are now available[16–18].

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COMMENTARY

This vision of structural biology and biomedical research is almost 14 years old. Reexamining 'old visions' in the light of new knowledge may be instructive.

– Editors

Looking back: A vision of molecular science and medicine*

V. Ramalingaswami

There is, today, a new and beautiful vision of life revealed to us by modern biology. The vision is linked to a hope of being able to cope with the problems of *population growth, agriculture* and *parasitic diseases*. Understanding molecular structure and relating it to biological function is today the most exciting and intense pursuit of science. So far as medicine is concerned, at first it was correlating altered morphology of organs in disease states with clinical syndromes. This could be called the Rokitansky era led by the Vienna school. With the discovery of the light microscope, this pursuit of relating structure to function reached its culmination in the concept of *cellular pathology* propounded by Rudolf Virchow. Virchow called the cell as the *theatre of life*. The pioneering work of Pasteur and

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Koch a hundred years ago identified microbial causation of disease which led to the widespread use of sanitation, hygiene and immunization. As Lederberg has said, the second revolution since 1950 has resulted in a profound understanding of cell biology, of microbes and viruses. DNA stood out as the symbol of this work. Now the third cycle, which is in progress, is revealing the possibilities of practical benefits of DNA-oriented research.

With the development of biochemical and biophysical techniques including ultrastructure, we have now reached the most exciting phase of molecular structure in relation to biological function. Molecular and cell biology have already given us deep insights into the structure and organization of the constituents of life processes and we will see a much more elaborate and sophisticated picture of the chemistry of life.

Today's symposium deals largely with the structure, conformation and interaction patterns of biomolecules. This is the centre piece of the biological revolution we are in. This is not just accretion of new data; it is a revolution in science. It is today more exciting to read *Nature* and *Science* than *Le monde*. We have moved from the alpha helical model for polypeptides proposed by Linus Pauling in the fifties, through the Watson–Crick double helical model of DNA, through the triple helical model of G. N. Ramachandran and Rich and Crick for collagen. Ramachandran and his colleagues also put model building on a more quantitative basis by introducing the concept of torsional (conformational) analysis. The spectacular X-ray crystallographic studies on a variety of protein crystals supplemented more recently by several other spectroscopic, synthetic, physical, chemical and biochemical techniques have proved to be extremely useful in biophysical analysis. In depth analysis of nucleic acids, proteins, polypeptides, oligonucleotides, polysaccharides, biomembranes, many multi-molecular assemblies are all coming under systematic attack through experimental and theoretical techniques.

Underneath a great mass of complex and confusing detail, there are great discoveries which are simple. Kenneth Boulding called the Biological Evolution more poetry than science. There is chance and chase in Discovery and Creativity.

Understanding the structure of DNA has contributed to many aspects of microbiology and public health. We now have considerable information on the organization and activity of microbial DNA and recombinant DNA technology aided by the selective use of monoclonal antibodies will result in the development of new vaccines. Sub-unit vaccines will overcome some of the problems associated with killed and attenuated vaccines such as the hazards of causing the disease itself, temperature instability and lack of protective effect against all the antigenic variants of the pathogens. The purity of the subunit vaccines and absence of the pathogen's genetic material are further advantages. Recombinant DNA technology can produce a part of the surface protein molecule of the pathogen while chemical synthesis of short polypeptide that represents surface proteins can also be accomplished (molecular cartography). Take the case of cell membrane surfaces and how they interact with their environments and with other cells. What is the basis of recognition? Vaccines against viral diseases now constitute a major thrust and hepatitis B subunit vaccines illustrate the developments in this field. Genes that encode portions of the hepatitis surface antigen have been cloned and a subunit vaccine from recombinant DNA in yeast is now under trial. Chemical synthesis based on known amino acid sequences of virus surface proteins and peptide sequences likely to elicit immune responses are being identified. Fairly small amount of protein may be sufficient, a few kilograms, for immunizing millions. The future alone will tell us as to whether microbial bioprocess route or chemical synthetic route would be optimal. It is also possible to develop multivalent vaccines that protect against several diseases in a single preparation by combining a number of peptide sequences to elicit responses for several different antigens and thus broaden the spectrum of synthetic subunit vaccines. Large carrier proteins are being employed to improve the immune response. A live virus vector system using the vaccinia virus which is very stable at room temperature is being investigated and already ten different protective antigens have been shown to 'piggyback' on the vaccinia virus successfully. These are: Hepatitis B surface antigen, circumsporozoite protein of malaria parasite, Herpes simplex, Rabies, Influenza, Epstein-Bar virus. DNA encoding, for example, for hepatitis B surface antigen is joined to DNA sequences which control transcription of the surface antigen DNA and this recombinant DNA construct is inserted into the vaccinia virus and a living vaccine that synthesizes and secretes surface antigen is produced.

The sporozoite surface protein has been put into vaccinia virus, the virus expresses the protein and the animals are immunized. *Malaria vaccine will be the first vaccine against parasitic disease and the first pay-off from molecular biology against parasitic disease.*

This is a resurrection of vaccination with vaccinia virus, using it as a 'donkey' to carry and express a wide variety of immunogens.

From viruses to bacteria and to parasites we encounter increasing complexity and yet modern biology's greatest triumph might well be in the breakthrough that it might provide in immunizing populations against parasitic disease. Until recently, difficulties in growing the parasites *in vitro*, their complex life cycles, their ability to change their spots and evade the body's immune system through antigenic variations and the generally poor state of knowledge on the immunobiology of parasitic infections hampered progress. But in recent years there is a sea change in the situation. Again it is the surface antigens that are proving to be crucial in the fight against parasitic diseases. Recombinant-DNA-produced surface antigens like the circumsporozoite protein of malarial parasite offer great promise for a protective malaria vaccine. The ultimate goal here is to produce a cocktail of a number of stage-specific antigens that will act as a defence system that will protect against infection, neutralize the asexual forms and prevent transmission through the mosquito.

The circumsporozoite protein is a simple antigen, has only 4 amino acids – proline, asparagine, alanine and again asparagine, repeated 23 times without variation; it has a molecular weight of 58,000, holds the key to the malaria vaccine. The antigen is encoded in a single gene and does not require any

alteration to render it antigenic. Five groups in the world achieved expression of cloned genes encoding sporozoite and blood stage proteins of *P. falciparum*.

Four of the antigens contain short repetitive sequences. The significance of this may be in generating antigenic diversity to evade host immunity.

Tuberculosis and leprosy are still rampant in most parts of the developing world in spite of the availability over the years of a number of chemotherapeutic agents against them. The identification of specific antigens, antigens specific to these mycobacteria, that will be strongly immunogenic and their production through recombinant DNA technology offers considerable scope.

Even simple organisms have a large and complex component of DNA. The tools we now have for the analysis of individual genes provide new ways of making peptide hormones, new drugs, diagnostic reagents.

Somatostatin, human insulin, human growth hormone, interferons have been produced in this manner. There is today a growing list of polypeptide products extending into large and extensively-folded proteins.

Human serum albumin which is of substantial molecular size, with 585 amino acids, has been produced through recombinant DNA technology. Factor VIII will soon be produced through gene cloning, which is even more challenging due to the extremely large size of the molecule (300,000 molecular weight). It now costs 1 million dollars per g, derived from normal human blood with the attendant risks of the AIDS virus and the delta hepatitis virus. Delta hepatitis is caused by a delta virus piggybacking on another virus, the hepatitis B virus. This raises the question of viral combinations in human disease.

DNA of course lies at the heart of the cancer problem and much of the work on carcinogenesis is devoted to the interaction of carcinogens with DNA and its genetic consequences. There is a central role for DNA in the understanding of the development of cancer and in the scientific basis of non-surgical treatment of cancer such as radiotherapy and the use of alkylating agents to treat cancer which act by affecting the DNA. There is so much of excitement in cancer research today that the editor of *Nature* was carried away and predicted that the genesis of cancer would be finally understood in 1983 itself. *Oncogenes* are of course on everybody's lips. They are present in normal cells and in the process of carcinogenesis, they change in their structure or expression. 30 or so key genes have so far been involved in the genesis of cancer. Normal genes can be transformed into cancer genes or genes or gene segments derived from viruses may 'break into' human genes and make them behave as cancer cells – lack of adhesiveness, and eternal division. Philip Leder at Harvard described work in which a normal gene was turned on by a viral regulator–molecular switch and produced cancer by a change in timing and extent of the normal gene's activity. Perhaps we can conceive of 50 genes out of our complement of 130,000 genes that could be functionally involved in the neoplastic phenotype. It is our ability to manipulate DNA that has now brought together the disciplines of cytogenetics, chemical carcinogenesis and viral oncology into a holistic approach to carcinogenesis. Out of this bewildering array of knowledge of normal genes and their neoplastic counterparts will emerge useful knowledge of how cell growth, cell differentiation, function and behaviour are controlled. Of course the proteins encoded by the oncogenes which seem necessary to malignant behaviour have become targets for chemotherapy offering specific sites for interference with chemotherapy without affecting normal cells – the ideal chemotherapeutic agent, the 'magic bullet' of Paul Ehrlich. What are the functions of proteins encoded by oncogenes anyway?

Advances are being made with cytochemical techniques, using oncogene-specific antibodies and nucleic acid probes to study oncogene expression. Perhaps there will be a molecular classification of cancer to replace the present pathological classification. The analysis of gene rearrangement in B-cell and T-cell tumours is leading to a new classification of lymphomas and leukemias.

While this explosion of knowledge of molecular biology of cancer may find application within the next few years, trophoblast sampling and restriction enzyme analysis of foetal DNA in the first trimester of pregnancy now enables prenatal detection of genetic abnormalities such as abnormal haemoglobins and replacement of defective genes by normal genes offer a possibility of cure in the distant future. Retrovirus vectors are proving to be efficient in integrating genes into haemopoietic stem cells. Enzyme deficiencies can be corrected in the same way in genetic enzyme deficiencies through gene replacement and this will probably happen earlier than gene replacement for abnormal haemoglobins.

The synthetic steroid which binds to the progesterone receptor and hinders its activities provides an important contraceptive ('contragestive') effect which can be obtained by means of antiprogesterone action as soon as fertilization has occurred (Baulieu). Progesterone is essential for the transformation of endometrium from the proliferative stage to the secretary stage which permits the implantation of the embryo. The progesterone receptor displays a cavity adapted to the shape and structure of the hormone (*Editors' note: For a detailed view of the interaction of progesterone with its receptor as revealed by X-ray crystallography, see S. P. Williams and P. B. Sigler, Nature, 1998, 393, 392*). When this binding site is obstructed by introducing a molecule that resembles the natural hormone, the access of progesterone is blocked without activation of the receptor. In other words a 'wrong key' has been inserted and the door cannot be opened.

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